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Induction of Extra Claws on the Chelipeds of a Crayfish, *Procambarus clarkii*

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Abstract. In a crayfish, *Procambarus clarkii*, growth of an extra claw was induced by making a V-shaped wound in the proximal end of the propodus of the second and third chelipeds. Two nerve bundles were damaged by the wounding. Some type of extra growth developed on the propodi of 13 of the damaged chelipeds: a pair of extra claws (2 chelipeds), a pair of extra dactyls (1 cheliped), a single extra dactyl (2 chelipeds), and a single slight projection (8 chelipeds). The extra claws and dactyls developed from the peripheral side of the propodus away from the wound site. One of a pair of extra dactyls and the single extra dactyls could be moved only slightly, either manually or by the crayfish. The other extra dactyls could be moved by the crayfish. Muscles were associated with each of the extra and primary claws. The muscles attached to the double extra claw or dactyl were innervated by nerve bundles that were branches from the primary thick nerve bundles. One possible explanation for these findings is that the severed nerve fibers in the thick nerve bundles regenerate, elongate into aberrant roots, and form extra claws or dactyls.

Introduction

The occurrence of double claws has been observed in the past. They have been reported to occur naturally on the cheliped of the American lobster, *Homarus americanus*, from the primary propodus near the dactyl (Faxon, 1881) and from the base of the primary propodus (Cole, 1910). In the crayfish, *Procambarus clarkii*, extra claws have been reported to occur naturally on the first and third chelipeds (Nakatani *et al.*, 1997). Both of these extra claws developed from the base of the primary propodus. These crayfish were not able to move the dactyls of the extra claws, although they could be moved manually. Nerve bundles were observed to branch from the primary thick nerve bundles into the extra claw on the third cheliped (Nakatani *et al.*, 1997).

Lateral outgrowths on the first cheliped of crayfish can be induced by wounding. In one study (Murayama *et al.*, 1994), outgrowths developed in about 10% of wounded chelipeds. This percentage was increased to 61.9% by increasing the width and depth of the wound and removing the tissue in the wounded area (Nakatani, 1996). Many of these outgrowths have serrations and hooks. However, there is no articulation between the primary propodus and the new structure. These structures, hereafter called "lateral outgrowths," may be distinguished from an extra claw or extra dactyl by the absence of an articulation.

The objectives of the present study were to induce the growth of extra claws and to investigate the degree of movement and innervation of the extra growth.

Materials and Methods

The experimental crayfish

Crayfish (*Procambarus clarkii*) of both sexes were collected, without regard to molting stage, from ponds in the suburbs of Yamagata City, Yamagata, Japan. The length of the carapace varied from 15.2 to 36.6 mm. We used both intact and eyestalk-ectomized specimens because eyestalk removal promotes growth and decreases molt interval (Fingerman and Fingerman, 1974; Nakatani and Otsu, 1979; 1981; Suzuki, 1980).

Wounds

The dorsal side of the base of the propodus of the second and third chelipeds was wounded using small scis-

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sors. The V-shaped incision was made to a depth of about half the width of the base of the propodus (Fig. 1). The ventro-median tissues were destroyed by inserting a needle (1 mm in diameter) into the wound. In 34 crayfish, a total of 134 chelipeds were wounded. Both eyestalks of five of the crayfish were removed from the base 10 to 20 days post-operation.

Rearing wounded crayfish

From September 1996 to March 1997, the wounded crayfish were reared until they had molted two or three times. The animals were kept separately in individual containers (300 × 240 × 105 mm) under a natural photoperiod at room temperature (18°–26°C). Prawn pellets (Super B; Nihon Nosan Industry, Japan) and dry persimmon leaves were placed in the rearing containers so that the crayfish could feed on them *ad libitum*.

Sensory neuron responses

A cheliped with extra dactyls was cut off at the base of the merus and held on a glass slide by rubber bands. The exoskeleton at the base of the merus was removed and the nerve bundles were exposed. To detect responses from the sensory neurons, the point of a needle was used to push the extra dactyls in a closing direction. Action potentials from the exposed nerve bundles were recorded extracellularly, using suction electrodes.

Mechanical force exerted by closure of dactyls

Measurements were taken on the force exerted by closing the dactyls. The cheliped with double extra dactyls was cut off at the base of the merus and prepared as described above. Nerve bundles were stimulated using a pair of tungsten hook electrodes. Repetitive pulses (single pulse, 10 ms in duration; 20 Hz) were applied for 0.5–2 s to hook electrodes connected to an electronic stimulator (SEN1101; Nihon Kohden, Japan). The mechanical force exerted by closure of each of the two dactyls was recorded simultaneously with a thermal array recorder (TRA1100; Nihon Kohden) *via* a high-gain force-displacement transducer (SB-1T-H; Nihon Kohden) coupled mechanically to the tip of the dactyl by a silk thread filament.

The sensory nerve responses and mechanical force were recorded at room temperature (23°-28°C).

Observations on nerve bundles and muscles

Observations were made on the nerve bundles and muscles of the propodus. The exoskeletons of the propodus and carpus of the above-mentioned chelipeds were removed, and nerve bundles and muscles were exposed and placed in physiological solution for crustaceans (van Harreveld, 1936). The nerve bundles were stained with a 0.005% solution of methylene blue dissolved in physiological solution and were observed under a dissecting microscope. The stained chelipeds were then fixed overnight at 0°C in a 20% formalin solution acidified to pH 3.8 with an acetate buffer. The muscles were observed with a dissecting microscope and a polarizing light microscope (BHSP, Olympus, Japan).

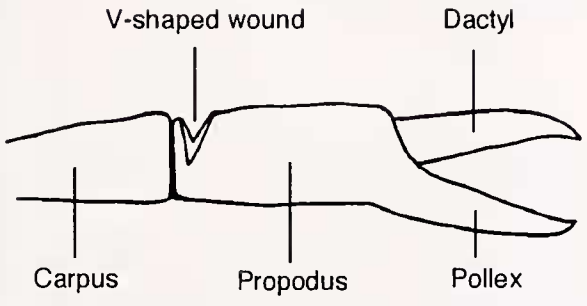

Figure 1. Diagram of the second cheliped of the crayfish showing V-shaped wound at the base of the propodus.

Results

Development of extra structures

Data on extra structure development are shown in Table I. Most of the chelipeds healed normally, but 13 (9.7%) formed extra structures. A pair of claws, a pair of dactyls, and single dactyls developed from the side away from the wound, and single, slight projections developed at the wound. These projections ranged in size from 0.3 to 1.8 mm at the second or third post-operation molt. Because extra structures developed at such a low frequency, we cannot draw any conclusions about the effect of eyestalk removal on their development.

Morphology of extra claws

A pair of extra claws that developed on one of the damaged chelipeds is shown in Figure 2. Here, the dactyl and pollex were torn off at the first post-operation molt. However, a pair of projections 0.6-mm long developed on the remaining untorn part (arrowheads in Fig. 2A). Two extra claws appeared on the propodus near the primary dactyl, and the primary dactyl and pollex regenerated at the second molt. The lengths of the three dactyls (Fig. 2B, a, b, c) and the single contralateral dactyl were 0.5, 1.9, 1.7, and 3.3 mm, respectively.

The three claws elongated at the third molt, and the shape of each claw was normal. There was no boundary among the propodi of these claws. The lengths of these three dactyls (Fig. 2C, a, b, c) and the single contralateral dactyl were 2.9, 3.7, 3.7, and 4.4 mm, respectively.

Table I

Summary of wounding experiment in the crayfish Procambarus clarkii *(values represent number of chelipeds)*

			Developed extra structures[3]				
			Double		Single		
	Wounded[1]	Autotomized[2]	Claws	Dactyls	Dactyl	Projection	Healed normally[4]
Eyestalks							
With	114	10	1	1	1	8	93
Without	20	1	1	0	1	0	17

[1] A total of 134 chelipeds from 34 crayfish received a V-shaped incision. After 10 to 20 days, both eyestalks were removed from 5 of the animals (20 chelipeds).

[2] Some animals autotomized chelipeds post-operatively.

[3] Extra structures developed in 13 chelipeds.

[4] Most of the chelipeds healed normally, with no outgrowths.

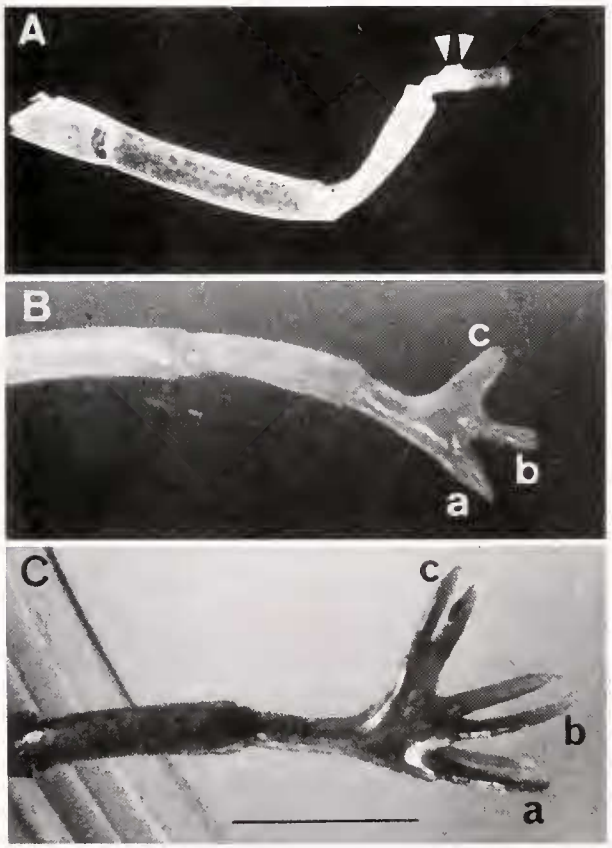

Figure 2. Development of extra claws on the third cheliped of the crayfish after a wound was made in the propodus. Both eyestalks were removed at the base on day 11 after wounding. (A) Two projections 0.6-mm long (arrowheads) developed on the propodus, but the dactyl and pollex were torn off at the first post-operation molt. (B) The projections developed into two poor claws at the second molt, and the untorn remaining part regenerated the dactyl and pollex. (C) The claws developed fully at the third molt. A and B, the castoff exuviae after the second and third molts, respectively. a, primary claw; b and c, extra claws. Bar = 5.0 mm.

Morphology of a pair of extra dactyls

Two dactyls appeared on one of the chelipeds of a crayfish with eyestalks. The V-shaped wound seemed to have healed normally at the first molt. However, extra dactyls appeared on the right second cheliped at the second molt (Fig. 3, a, b). The extra dactyls developed on the primary propodus near the primary dactyl, although the wound was made at the base of the propodus. The primary claw was angled about 45° to the left of the propodus near the primary dactyl. The angle of the extra dactyls and the primary claw was about 90°. The extra dactyls a and b of Figure 3 are hereafter called "extra dactyl a" and "extra dactyl b," respectively. The lengths of the carapace, extra dactyls a and b, and primary and contralateral dactyls were 39.5, 2.9, 3.1, 5.1, and 3.1 mm, respectively.

Morphology of single extra dactyl

A single extra dactyl that developed on the propodus of the second right cheliped at the second molt is shown in Figure 4. The extra dactyl was located between the base of the primary propodus and the base of the primary dactyl. The extra and primary dactyls were 2.0- and 3.7-mm long, respectively.

Movement of the extra and primary dactyls

In the example shown in Figure 2, the crayfish could move the primary dactyl but not the two extra dactyls at the second molt. Extra claw b was usually in an open position, but opened readily when it was closed manually. After the third molt, the crayfish could move the extra and primary dactyls. It always moved these three dactyls asynchronously and often spontaneously moved the primary dactyl synchronously with extra dactyl b. These

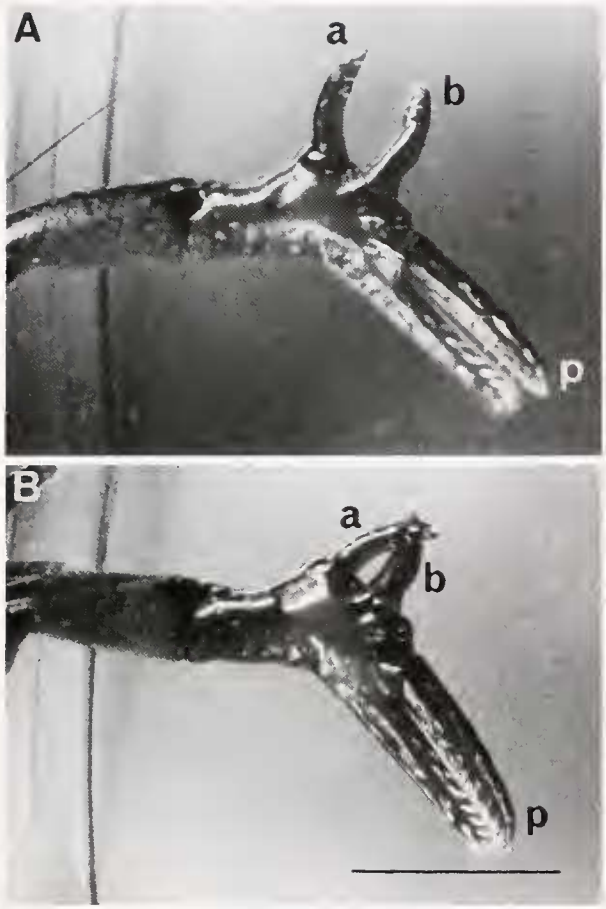

Figure 3. Two extra dactyls on the right second cheliped produced after the propodus was wounded. (A) Extra dactyls in the open state. (B) Extra dactyls in the closed state. a and b, extra dactyls; p, primary dactyl. Bar = 5.0 mm.

three dactyls would move synchronously when one of them was touched.

In the diagram shown in Figure 3, the crayfish could move the extra dactyls and the primary dactyl. When it was picked up, it moved the extra dactyl a more frequently than the primary dactyl. However, it moved these dactyls simultaneously if one of them was touched. The single extra dactyl in Figure 4 and extra dactyl b in Figure 3 could be moved only slightly, either manually or by the crayfish.

Muscles and innervation

Muscles were observed to correspond to each claw in the propodus. In view of their locations, these muscles could control the opening and closing of the three claws. The closer muscles are shown in Figure 5A.

Four muscles in the propodus with two extra dactyls (Fig. 3) were dissected out as shown in Figure 6B. In this figure, muscles m1, m2, m3, and m4 are the closer muscle of the primary dactyl, the closer muscles of extra dactyls b and a, and the opener muscle of extra dactyl a, respectively. The mean (± standard error of the mean) lengths of 10 sarcomeres of each of these muscles after being fixed with formalin solution were 6.4 ± 0.3, 6.2 ± 0.3, 7.7 ± 0.4, and 5.8 ± 0.5 μm.

There were two thick nerve bundles in the intact propodus of the second cheliped (Fig. 6A): one ran toward its dactyl along the boundary between the opener and closer muscles; the other ran to its pollex along the closer muscle. The extra dactyls shown in Figure 3 were innervated with nerve bundles from the primary nerve trunks. A thick nerve bundle ran toward the primary dactyl between the two closer muscles (Fig. 6B, m2 and m3) of the extra dactyls. At the proximal part of the propodus, the nerve bundle ran between the closer muscle of the primary dactyl (m1) and the opener muscle of the extra dactyl a (m4) (Fig. 6B, C).

In a propodus with two extra claws, the nerve bundles branched to each claw from the primary nerve trunk (Fig. 5B).

Extracellular recording

An extracellular recording was made from the nerve bundle at the merus of the cheliped with two extra dactyls. The neuron in the nerve bundle responded to the push toward closing of extra dactyl b (Fig. 7). However, the neuron did not generate action potentials when the dactyl moved in the opposite direction. Therefore this response might be the action potential from proprioceptors.

Mechanical force generated by dactyl movement

The mechanical force generated by the movement of the primary and extra dactyls in response to electrical

Figure 4. A single extra dactyl that developed on the right second cheliped. Both eyestalks were removed on day 12 after the cheliped was damaged. The arrowhead shows an articulation. Bar = 5.0 mm.

stimulation of the nerve bundle in the merus was measured. The force generated by the primary dactyl was about 8- and 3-fold greater than that generated by extra dactyls a and b, respectively (Fig. 8). The primary dactyl moved spontaneously and asynchronously with the extra dactyls just before the electrical stimuli were applied to the nerve bundles (Fig. 8A, A′).

Discussion

There have been previous reports of double extra claws developing from the carpus (Bateson, 1894) and coxa (Przibram, 1921) of the crayfish (*Astacus fluviatilis*) and from the propodus of the cheliped of lobster (*Homarus americanus*) (Cole, 1910). Recently, two extra claws that developed naturally in *P. clarkii* were described (Nakatani *et al.*, 1997). These single claws were borne on the first and third chelipeds—one on each propodus. The extra

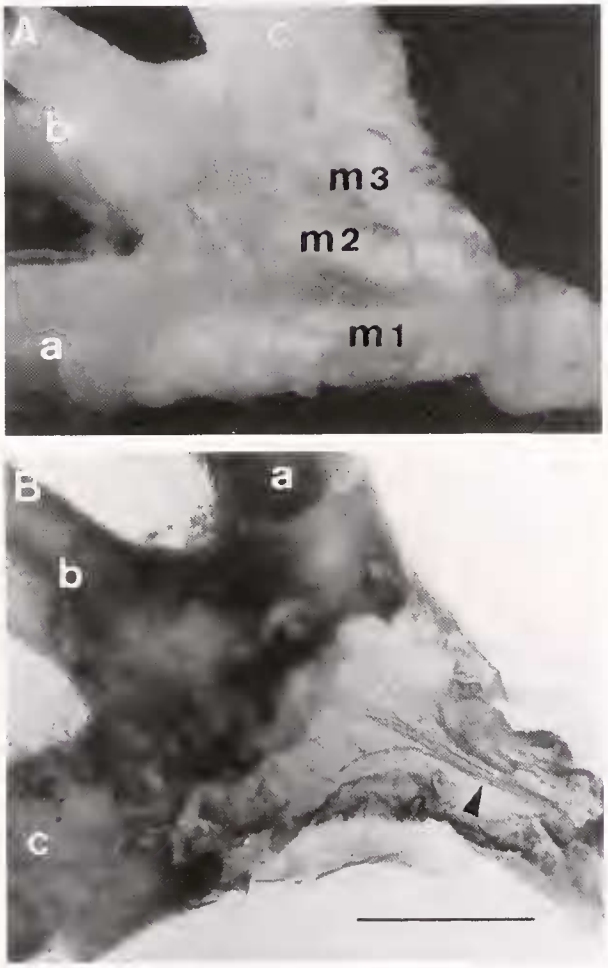

Figure 5. Innervation and muscles in the propodus of a cheliped with two induced extra claws. The cheliped is the same as that shown in Figure 2C. (A) Muscles corresponding to each claw (dorsal view). (B) The nerve trunks in the primary propodus branch, and each branched nerve bundle innervates a claw (ventral view). a, primary claw; b and c, extra claws; m1, m2, and m3, closer muscles associated with the primary claw (a) and extra claws (b, c), respectively. Arrowhead shows the nerve trunks. Bar = 1.0 mm.

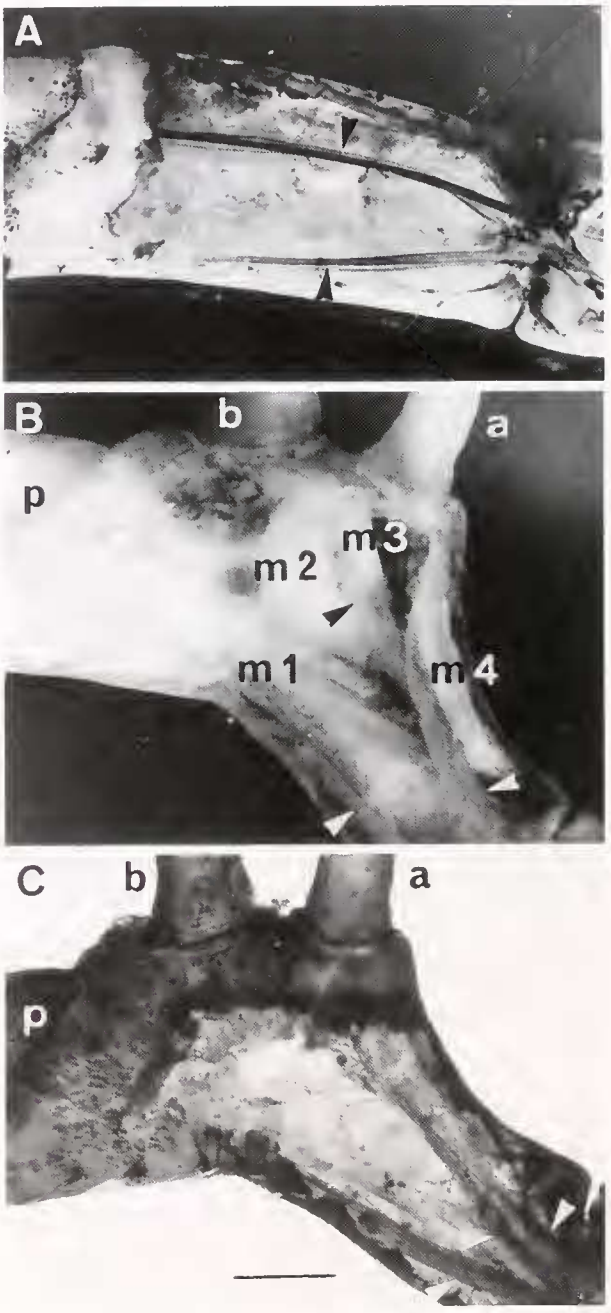

Figure 6. Innervation and muscles in a propodus with double extra dactyl and in a normal cheliped. The cheliped with extra dactyls is the same as that shown in Figure 3. (A) Two nerve bundles (arrowheads) in the propodus of the second cheliped. (B and C) Branched nerve bundles from the primary nerve trunks (arrowheads) innervate the extra dactyls. m1, closer muscle of the primary claw; m2 and m3, closer muscles of the extra dactyls b and a, respectively; m4, opener muscle of the extra dactyl a. a, b, and p are extra dactyl a, extra dactyl b, and primary dactyl, respectively. Bar = 1.0 mm.

Figure 7. Extracellular recording from nerve trunks in the merus of a cheliped with extra dactyls. Thick bar signifies manual closing of the extra dactyl shown in Figure 3.

claws induced in the present study are essentially similar to those naturally occurring ones. The naturally occurring extra claws reported by Cole (1910) and Nakatani *et al.* (1997) developed from the base of the primary propodus. Lateral outgrowths from experimental incisions developed from the wounded site on the outer surface of the propodus (Murayama *et al.*, 1994; Nakatani, 1996). Thus, in the present study, the wound was made at the base of the propodus. However, all of the extra claws or extra dactyls developed on the distal side of the wound. This suggests that the extra claws reported by Cole (1910) and Nakatani *et al.* (1997) were caused by damage at a site more proximal to the extra claw—for example, at the carpus.

Although there were no extra propodi, the shape and location of the a pair of extra dactyls observed in this study were similar to those of the naturally occurring one reported by Faxon (1881) on the cheliped of *H. americanus*. As in the present study, these extra claws developed away from the base of the propodus. The propodi of the extra claws described by Faxon were about one-sixth as long as the extra dactyls, and each was separated from its primary propodus by an articulation.

Lateral outgrowths on chelipeds have been assumed to result from the abnormal healing of a natural wound (Suzuki and Odawara, 1971; Shelton *et al.*, 1981; Okamoto, 1991; Nakatani *et al.*, 1992). Many of those outgrowths consisted of a pair of projections without any articulation. The proximal and distal surfaces of the wound each developed one projection, and they faced each other as mirror images (Nakatani, 1996). However, in the present study, the extra dactyls developed away from the damaged site. Different mechanisms may be involved in the development of a lateral outgrowth and of an extra claw or dactyl. In the propodus it was observed that one nerve trunk runs in the direction of the pollex and another to the dactyl.

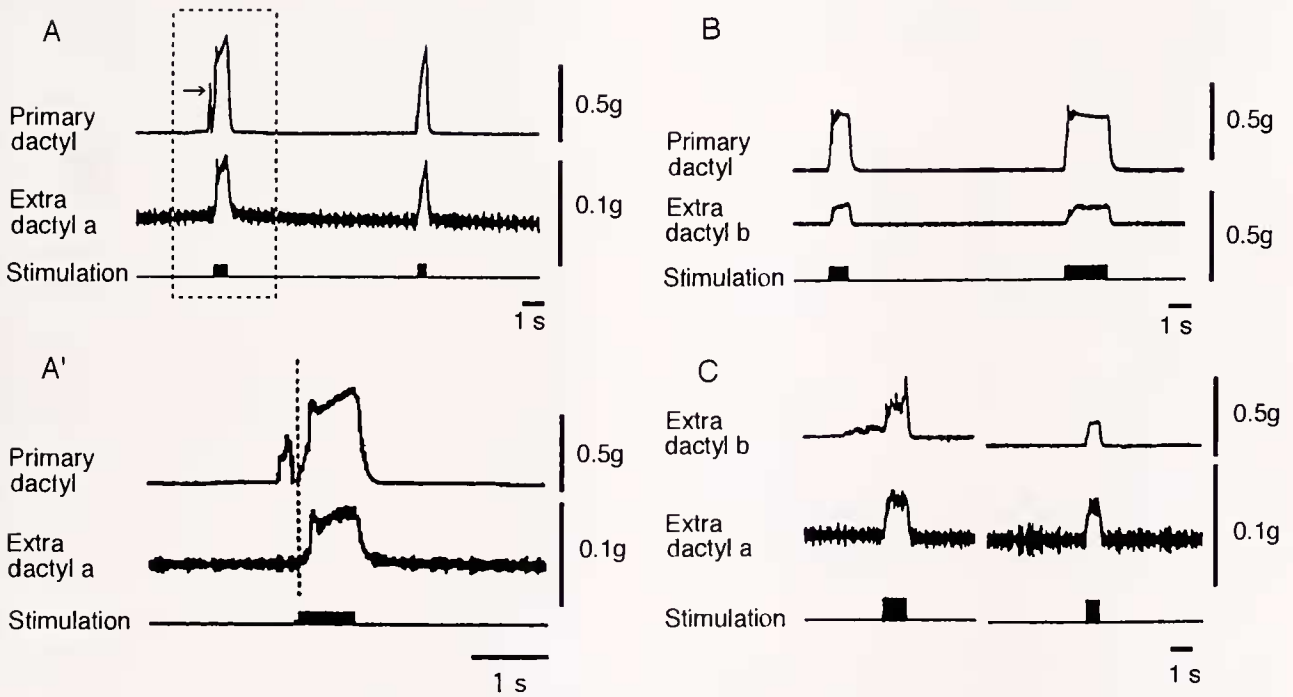

Figure 8. Movements of the primary and extra dactyls. The movements generated by each of two of three dactyls were recorded simultaneously when repetitive pulses (single pulse, 10 ms in duration; 20 Hz) were applied to the nerve trunks in the merus, which was monitored in each bottom trace. The same cheliped shown in Figure 3 was used. A', the time-scale for enclosed part with broken line in A was magnified 4-fold.

Lateral outgrowths were induced by wound damage of the nerve trunk that runs toward the pollex (Nakatani, 1996). Meanwhile, in the present study, both nerve trunks may have been damaged by wounding, because of the depth of the wound and the location of the nerve trunks.

The crayfish reported by Nakatani *et al.* (1997) could not move the dactyl of its natural extra claws, despite the presence of muscles and nerve bundles. In contrast, all of the extra dactyls in the present study could be moved by the crayfish. Further studies are needed to explain this difference, but some speculation is possible. Closer and opener muscles, and innervation of these muscles, are necessary for a crayfish to move its dactyls. The crayfish claw is moved by two muscles controlled by five efferent axons: one inhibitor and one excitor to the opener muscle, and one inhibitor and two excitors—a "fast" and a "slow"—to the closer muscle (van Harreveld and Wiersma, 1937; Wiens, 1976). Both the primary and extra claws can be functional if the axons are adequately branching and innervate the muscles of each claw. The primary and extra dactyls may move synchronously if the above-mentioned axons branch to each muscle. However, the crayfish observed in this study could move all three dactyls synchronously or asynchronously. Atwood (1973) reported that a wide variety of synaptic mechanisms and muscle fiber properties permit delicate control within a simple framework in the crayfish claw. The articulation of the dactyl also affects its mobility. An extra dactyl b could be moved only slightly by hand, even though it had well-developed closer and opener muscles, and innervation (Fig. 6B, C).

For an extra claw or dactyl to develop, some of the severed nerve fibers must separate from the primary nerve trunks. The present results suggest that severed nerve fibers regenerate; some of them elongate to form aberrant roots and extra claws or extra dactyls. Aberrant roots have been observed in the abdominal nerve cord of the crayfish *P. simulans* by Bittner *et al.* (1974). They reported that neurons projected aberrant roots toward the periphery after the third abdominal ganglion was removed. Also, Goransson *et al.* (1988) showed that in crayfish *P. clarkii*, the regenerating neurons would orient themselves in the proper direction to make connections toward the preferred target area. Further, the neurons do not respond to positional cues during the first week of regeneration.

Other studies have shown that transplanted tissues can induce growth of new structures. Kao and Chang (1996) proved that dactyl, pollex, and ischium tissues of the crab claw all had claw-transforming activity if they were autotransplanted into the autotomized stump of the fourth walking leg. Furthermore, by autotransplantation of a pollex into the eye sockets, a claw with complete proximal segments (ischium, merus, carpus and manus) developed (Kao and Chang, 1997). In the cockroach *Blattella germanica*, graft/host junctions of the leg regenerated segmented structures consisting of two copies of all structures distal to the point of the junction (French, 1976). French (1976) speculated that the graft and host do not heal together and interact, but rather regenerate autonomously in mirror-image symmetry of the original graft and host levels. Further studies are needed to explore related issues in crayfish.

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